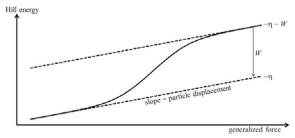
the complete dynamics is captured by the Hill energy = $kT\ln[(A-A_{min})/(A_{max}-A)]$. Although any marker of activation can be used, in practice the conductance Hill energy offers a large signal to noise ratio and has seen implicit use in ''limiting slope'' experiments. Hill plots theoretically yield ''model-free'' measurements of particle displacements and allosteric energies, but in practice discrimination between gating models can hinge on precise and sometimes difficult measurements of A_{min} and A_{max}. Illustrative examples using voltage-sensitive calcium channels and inward rectifiers will be provided.



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Domain-Specific Gating-Modifier Toxins for Voltage-Gated Calcium Channels

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Few gating-modifier toxins have been reported to specifically target T-type calcium channels, and the structural basis of toxin sensitivity remains incompletely understood. Unlike the homotetrameric Kv channels, voltage-gated calcium channels are comprised of four different domains, presenting the possibility of multiple toxin binding sites. Using chimeric constructs, we screened existing gating-modifier toxins against the putative paddle motif from each domain of T-type calcium channel, Cav3.1. This helix-turn-helix motif is a critical structure responsible for both voltage-sensing and toxin sensitivity in Kv channels. We found that the four individual paddle motifs of Cav3.1 channels display unique toxin binding capabilities, suggesting that gating-modifier toxins can bind to T-type calcium channels in a domain-specific fashion. For two known T-type gating-modifier toxins, kurtoxin and ProTx-II, we identified key acidic and hydrophobic residues necessary for toxin binding in Cav3.1/Kv2.1 channel chimeras. Mutations of these residues in the wildtype Cav3.1 channels reduce toxin sensitivity, providing critical information on the binding sites of gating-modifier toxins in T-type calcium channels.

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The $\alpha_2\delta$ Subunit Facilitates $Ca_V1.2$ Channel Activation by Remodeling Its Four Voltage Sensor Domains

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Voltage-gated L-type calcium channels ($Ca_V1.2$) are multimeric proteins composed of the pore-forming α_{1C} subunit and at least two ancillary subunits, β and $\alpha_2\delta$, in a 1:1:1 stoichiometry. The $\alpha_2\delta$ subunit is a large extracellular protein with a membrane-anchoring segment. This auxiliary subunit not only promotes channel trafficking to the plasma membrane, but also facilitates channel activation, shifting the voltage dependence of channel opening toward more negative potentials. The underlying mechanism by which the $\alpha_2\delta$ subunit facilitates $Ca_V1.2$ activation is unknown. Using the voltage clamp fluorometry technique, we fluorescently tracked the molecular rearrangements of the four individual $Ca_V1.2$ voltage-sensing domains (VSDs) in the presence or in the absence of the $\alpha_2\delta$ subunit. We found that this subunit facilitated the activation of all VSDs, as revealed by a more hyperpolarized voltage dependence of VSD activation (FV), although to a different extent: the FV shift was ~-33 mV for VSD I, ~-19 mV for VSD II, ~-16 mV for VSD III and ~-96 mV for VSD IV, resulting in ~0.3 probability of VSD IV activation at resting potential (−80 mV). The $\alpha_2\delta$ subunit also shifted the voltage dependence of the conductance (GV) towards more hyperpolarized membrane potentials (~-70 mV). That is, the presence of $\alpha_2\delta$ subunits profoundly remodels the voltage sensor domains of $Ca_V1.2$ channels, facilitating voltage dependent activation. This facilitation emerges from distinct functional interactions between the $\alpha_2\delta$ subunit and individual $Ca_V1.2$ VSDs, consistent with $\alpha_2\delta$ asymmetric assembly with the α_{1C} subunit. The energetic contribution of each VSD to pore opening was quantified using linkage analysis combined with an allosteric model that consists of five gating particle (one pore and four VSDs). Funded by: NIH, AHA, FONDECYT, ACT.

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A Population Density and Moment-Based Approach to Modeling Domain Calcium-Mediated Inactivation of L-Type Calcium Channels

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We present a population density and moment-based description of stochastic domain calcium-mediated inactivation of L-type calcium channels. Our approach accounts for the effect of heterogeneity of local calcium signals on whole cell calcium currents; however, in contrast with prior work by Sherman et al. [Biophys J. 58(4):985, 1990], we do not assume that calcium domain formation and collapse are fast compared to channel gating. We demonstrate the population density and moment-based modeling approach using a12-state Markov chain model of an L-type calcium channel [Greenstein and Winslow, Biophys J. 83(6):2918, 2002]. Simulated whole cell voltage clamp responses yield an inactivation function for the whole cell calcium current that agrees or disagrees with the classic result of Sherman et al. when domains dynamics are fast or slow, respectively. We analyze the voltage-dependence of calcium inactivation that occurs via slow heterogeneous domains and find that when channel permeability is held constant, calcium inactivation increases as the domain time constant increases. However, when this parameter study is repeated for fixed maximum domain calcium concentration, inactivation decreases as the domain time constant increases. Comparison of simulation results using population densities and moment equations confirms the computational efficiency of the moment-based approach, and enables the validation of several distinct methods of truncating and closing the open system of moment equations. In general, a slow domain time constant requires higher order moment truncation for agreement between moment-based and population density simulations.

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Minimized Cell Usage for Stem Cell-Derived and Primary Cells on an Automated Patch Clamp System

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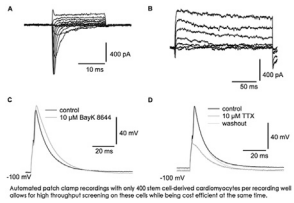
Automated patch clamp systems are widely used in drug development and safety pharmacology. The merits of automation generally come at the cost of large amounts of cells needed. While cell usage is of little concern when using standard cell lines such as HEK/CHO cells, it becomes a crucial constraint with cells of limited availability, such as primary or otherwise rare and expensive cells, like induced-pluripotent-stem (IPS) cell-derived cardiomyocytes or neurons.

We established protocols for CHO cells, IPS cell-derived neurons, cardiomyocytes and pancreatic islet cells, minimizing cell usage for automated patch clamp recordings on Nanion's Patchliner. We present a new cell application procedure achieving > 80% success rates for using as little as 300 to 2000 cells per well depending on cell type. We present recordings for these cell types, demonstrating that high data quality is not compromised by altered cell application.

Compared to other standard automated patch clamp systems we reduced the average amount of cells needed by more than 150 times. Reduced cell usage improves cost efficiency for expensive cells and opens up automated patch clamp for primary cells of limited availability.



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Testing for Direct Interactions Between the DHPR and the RyR1 Cytoplasmic Foot

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In skeletal muscle, RyR1 (5,037 residues) releases calcium from the sarcoplasmic reticulum (SR) in response to an orthograde signal from the DHPR in the plasma membrane (PM), and transmits a retrograde signal which increases the L-type calcium current via the DHPR (which contains $Ca_V1.1$ as its principal subunit). Previously, we tested the behavior of a RyR1 construct